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(54) Title: MEMBRANE SEPARATION PROCESS FOR PURIFYING, CONCENTRATING AND DENSIFYING ENZYME SOLUTIONS WITH POLYOLS

(57) Abstract: The present disclosure relates to methods and apparatuses for purifying, densifying and/or concentrating enzymes. In one preferred method, an enzyme preparation is brought in contact with a solution of water and polyol, thereby causing impurities to leave the enzyme preparation and causing increased density and/or concentration of the enzymes.

MEMBRANE SEPARATION PROCESS FOR PURIFYING, CONCENTRATING AND DENSIFYING ENZYME SOLUTIONS WITH POLYOLS

Background

The use of enzymes has increased in recent years through industrial, medical and domestic applications. Enzymes are naturally derived from several sources, such as plants, animals, bacteria, yeasts and fungi. Modern genetic engineering techniques have also widened the scope of enzyme applications.

In the areas of laundry and household care, enzymes are useful for aiding in the removal of a variety of stains, as well as fabric care. Suitable enzymes include, but are not limited to, proteases, amylases, lipases, cellulases, peroxidases and mixtures thereof. When choosing an enzyme for home and household care products, one typically considers one or more of the following factors: pH; activity and stability; thermostability; and compatibility with the other agents in the formulation.

Regardless of the type of enzyme or the intended use of the enzyme, the enzyme activity, concentration and density at the time of use or manufacture of product containing the enzyme will typically govern how much of the enzyme, i.e. on a wt% basis, is used or placed in the product. For example, if the activity of an enzyme preparation is high, less amount of enzyme preparation is needed to ensure the desired level of total activity in the final product, as compared to an enzyme preparation with a relatively lower

Summary

The present disclosure relates to methods and apparatuses for purifying, concentrating and/or densifying enzymes in solution (collectively "purifying" herein). In one preferred method, the enzyme preparation is placed in at least partial contact with a polyol solution, such as sucrose, propylene glycol, glycerol and, more preferably, sorbitol or combinations of polyols. The method allows for various impurities and water to leave the enzyme solution while leaving the enzymes behind and increasing polyol concentration in the enzyme solution. The purification process not only purifies the enzyme solution, thereby enhancing stability, but it also concentrates and densifies the enzyme solution. Small scale and large scale apparati are discussed for carrying out this method.

Brief Description of the Drawings

Figure 1 illustrates a preferred apparatus for purifying, concentrating and/or densifying enzymes; and

Figure 2 illustrates an alternate apparatus for purifying, concentrating and/or densifying enzymes.

Detailed Description

With reference to Fig. 1, apparatus 10 illustrates a preferred method of purifying, concentrating and/or densifying an enzyme preparation. Enzyme preparation 12 is disposed in partially permeable enclosure 14. Enzyme preparation 12 can be any commercially available preparation or laboratory preparation. Enclosure 14 is preferably fabricated from a membrane material that permits

TABLE 1

Enzyme Preparation	Before Processing		After Processing	
Protease	Activity GU/mg	Density g/ml	Activity GU/g	Density g/ml
Purafect	3,680	1.08	9,326	1.21
Properase	4,200	1.08	10,200	1.22
Lipase	Activity LU/mg	Density g/ml	Activity LU/g	Density g/ml
Lipolase	107	1.02	443	1.24

TABLE 2

Enzyme Preparation	Before Dialysis (Glycerol)		After Dialysis (Glycerol)	
Protease	Activity GU/mg	Density g/ml	Activity GU/g	Density g/ml
Properase 1600L	4,300	1.05	7,840	1.20

*Tables 1 and 2: Purafect and Properase are from Genencor, Lipolase is from Novo Nordisk.

Enzyme activity of protease and lipase was measured with a standard enzyme using casein and p-nitrophenylvalerate, respectively, as a substrate.

1. About 450g of enzyme preparation was placed into Spectra/Por® tubing (8,000 MWCO regenerated cellulose).

2. The tubing was placed in a beaker containing 3,500g of 70% sorbitol solution and mixed at room temperature.
3. After about 24 hours the tubing was removed. Density and activity were measured.

Continued operation would result in increased density and activity. Also, the sugar solution can be replenished to facilitate the process.

Other preferred operating parameters are as follows:

- pH: about 5 to about 10;
- temperature: any temperature that will not harm enzyme activity or stability and that will not cause difficulty of stirring solutions due to high viscosity;
- contact time: a couple of hours to days depending on desired density, activity and impurity level; and
- stirring: recommended to facilitate diffusion.

As can be seen in Table 1, activity levels/density levels increased 2.5/1.12 fold (Purafect), 2.4/1.13 fold (Properase) and 4.1/1.22 fold (Lipolase). As such, activity levels at least doubled and density levels increased by at least about 10%.

With reference to Fig. 2, an alternate purification, densification and/or concentration method and apparatus 100 is shown. Vessel 102 contains raw enzyme preparation 200 that can be pumped into enzyme reactor 110 by pump 108 through valves 104 and 106. Processed enzyme preparation 200' is removed from vessel 110 by pump 116 through valve

Claims

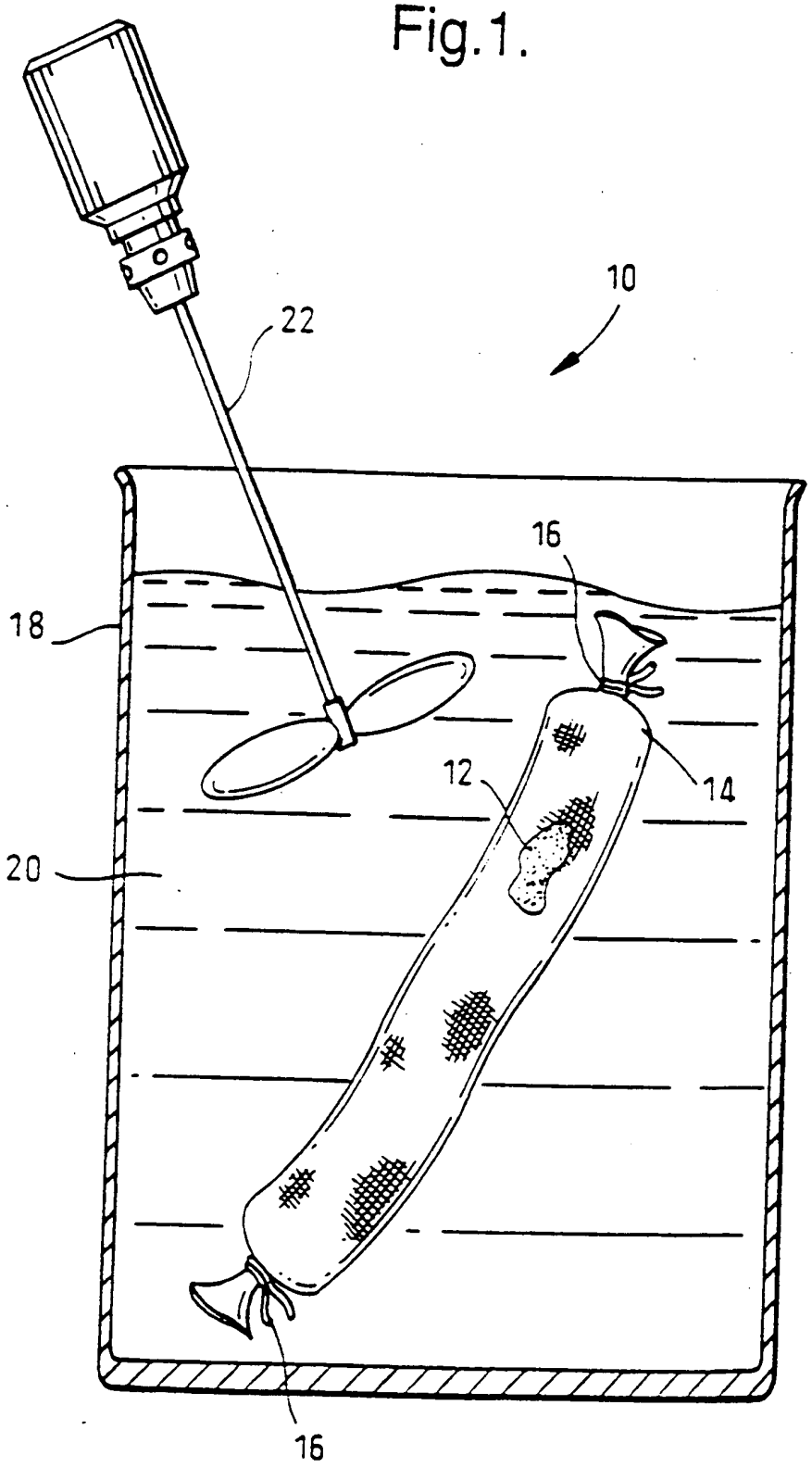
1. A process for purifying enzymes comprising:
   (a) preparing an enzyme an solution having at least one type of active enzyme, at least one type of enzyme impurity and at least water or solvent;
   (b) disposing the enzyme solution in an enclosure;
   (c) preparing polyol solution by dissolving at least one type of polyol in a liquid;
   (d) at least partially placing the enclosure containing the enzyme solution in the polyol solution;
   (e) allowing at least some of the impurity and water or solvent to leave the enclosure, thereby purifying and concentrating the enzyme solution; and
   (f) allowing at least some of the polyol to enter enzyme solution, thereby densifying the enzyme solution.

2. The method according to claim 1, wherein the step of preparing polyol solution comprises using sorbitol.

3. The method according to claim 1, wherein the step of preparing polyol solution comprises using glycerol.

4. An apparatus for purifying enzymes comprising an enclosure having first and second portions separated by a membrane, said first portion containing an enzyme preparation, said second portion containing a polyol solution.

Fig.1.



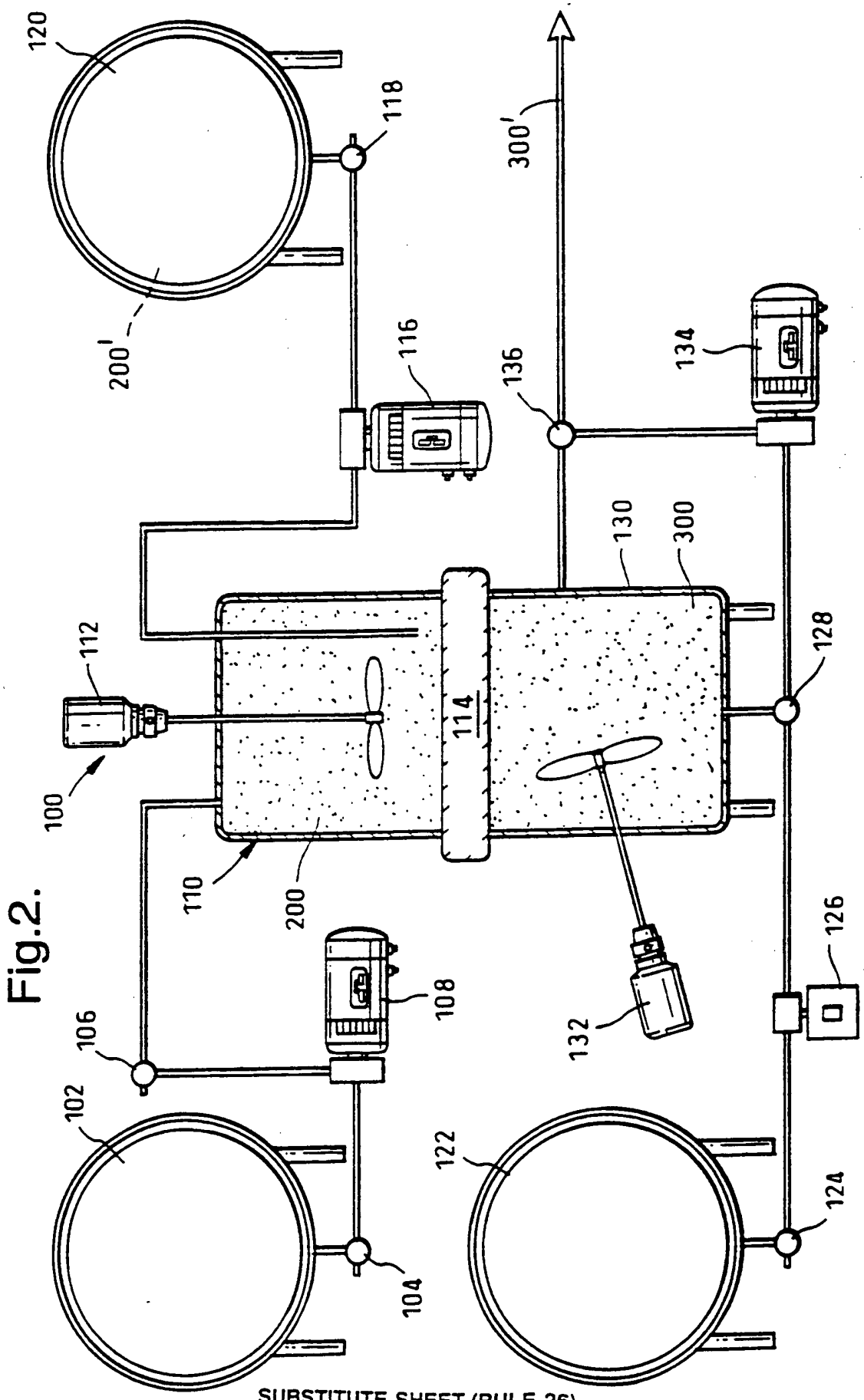

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Fig.2.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N9/00 C12M1/12 B01D61/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, CHEM ABS Data, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	US 5 354 669 A (KALUZA KLAUS ET AL) 11 October 1994 (1994-10-11) example 1 ---	1,3,4,6
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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INTERNATIONAL SEARCH REPORT

...ormation on patent family members

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PCT/EP 00/05908

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